Depressive-like States Heighten the Aversion to Painful Stimuli in a Rat Model of Comorbid Chronic Pain and Depression

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ABSTRACT

Background: Chronic pain and depression are two complex states with sensory/somatic and emotional components, and they may mutually exacerbate one another in conditions of comorbidity, leading to a poorer prognosis.

Methods: The authors have evaluated the sensory and emotional components in a rat model combining chronic constriction injury (CCI, a model of chronic neuropathic pain) with unpredictable chronic mild stress (CMS, an experimental model of depression). In addition, the phosphorylation/activation of the extracellular signal-regulated kinases 1 and 2 and neuronal density was also evaluated in the anterior cingulate cortex. Four groups were tested: sham-control, sham-CMS, CCI-control, and CCI-CMS.

Results: CMS selectively heightens aversion to painful experiences in animals subjected to CCI, as measured in the place escape/avoidance test at 20, 25, and 30 min (CCI-CMS (mean ± SEM): 75.68 ± 3.32, 66.75 ± 4.70, 77.54 ± 3.60 *vs.* CCI-control: 44.66 ± 6.07, 43.17 ± 6.92, 52.83 ± 5.92, respectively), in conjunction with an increase in the accumulation of phosphorylation/activation of the extracellular signal-regulated kinases (CCI-CMS: 4.17 ± 0.52 *vs.* sham-control: 0.96 ± 0.05) and a decrease in neuronal density in the anterior cingulate cortex. In contrast, chronic pain did not exacerbate the characteristic profile of depression (anhedonia and behavioral despair) in rats subjected to CMS. Furthermore, depression enhances the perception of some specific modalities of sensorial pain such as cold allodynia but has no influence on mechanical threshold.

Conclusions: These findings support the theory that depression leads to emotional dysfunction in the interpretation of pain in patients suffering chronic pain. In addition, combined animal models of pain-depression may provide a valuable tool to study the comorbidity of pain and depression.

What We Already Know about This Topic

- Pain and depression often coexist, yet whether they affect the pathophysiology of each other is unclear

What This Article Tells Us That Is New

- In rats subjected to a surgical nerve injury (model of neuropathic pain) and/or chronic mild stress (model of stress and depression), depression was associated with an increased aversion to stimulation of the hypersensitive area, but neuropathic pain did not alter behavioral assessment of depression
- These results suggest an important role for stress or depression on neuropathic pain and provide an interesting model for looking at their interlinked pathology

WHEN they occur concomitantly, chronic pain and depression are associated with a poorer prognosis than either condition alone, producing greater functional impairment of longer duration that responds more poorly to pharmacotherapy.[1,2] These observations are derived from epidemiologic data and they suggest that chronic pain and depression are reciprocally linked.[3] Indeed, their current definitions include several related components that can be identified as distinct constructs. As such, there is an experience sensory component of pain, which includes the perception of

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location, quality, and intensity of a noxious stimulus, as well as an emotional dimension, which processes the affective salience or unpleasantness of the noxious stimulus.[4] Depression is an affective disorder characterized by moods, feelings of worthlessness, diminished interest in pleasurable stimuli, and impaired decision-making abilities, although it can also have a somatic dimension characterized by weight change, fatigue, sleep disturbances, and pain.[5]

Although the sensory/somatic and emotional components of chronic pain and depression are well known, it remains unclear which are affected by their comorbidity and to what extent, hindering the development of effective pharmacotherapeutic and psychotherapeutic approaches. The general hypothesis from patient studies is that comorbidity provokes a general worsening in both conditions,[6,7] although the experimental data obtained from clinical populations is surprisingly diverse.[8] For example, the sensitivity to noxious stimuli applied to the skin may be normal or even reduced in patients suffering from depression,[9,10] even though they might show hyperalgesia in response to deep somatic pain.[11] Such inconsistencies may be partially explained by population heterogeneity, contextual factors, and the more specific nature of the disorders. Together, these methodologic limitations, some of which are inherent to clinical investigation, have considerably limited the study of comorbid pain and depression.

Because well-validated and controlled experimental animal studies have been carried out on pain and depression, we now have a valuable opportunity to advance the study of their comorbidity. There are well-established animal models of chronic pain that involve spinal nerve ligation and chronic constriction injury (CCI), both of which reproduce clinical sensorial symptoms of peripheral neuropathic pain.[12,13] More recently, new tools have been developed to evaluate affective processing of pain in rodents.[14,15] Although depression is considered a specifically human disorder, chronic mild stress (CMS) in rodents induces a depressive-like phenotype and this model has a high construct, face, and predictive validity.[16–18] Accordingly, we combined two well-established rat models of chronic pain and depression, CCI and CMS, respectively, to study the sensory-discriminative component of pain as well as the affective component (related or not to the pain experience). This specific experimental design allows us to better determine how patients suffering from chronic pain and depression encode the individual components of nociceptive input, and how mood is affected by pain.

Materials and Methods

Animals and Experimental Design

Experiments were performed using male Sprague-Dawley rats. All the experimental protocols were approved by the Committee for Animal Experimentation at the University of Cádiz, Cádiz, Spain and they complied with the International Association for the Study of Pain ethical guidelines.[19] All procedures relating to animal care and use conformed to European Ethical Standards (86/609-EEC) and Spanish Law (RD 1201/2005). The experimental design that was followed is illustrated in figure 1A. Briefly, animals weighing between 110–120 g were acclimatized to the housing conditions in groups of four for 1 week (acclimatization phase), after which they were caged individually for 3 weeks in the same room (habituation phase: H1–3). During these phases the animals were kept under standard laboratory conditions (water and food *ad libitum*, a constant room temperature of 22 ± 1°C and a 12-h light/dark cycle with lights on at 8.00 h). Later, animals (weighing 250–270 g) were subjected to CMS, an animal model of depression, and/or the surgical procedure to produce neuropathic pain through CCI (experimentation phase: E1 and E2). The animals were randomly allocated to the four experimental groups: sham-control (sham-operated without CMS), sham-CMS (sham-operated subjected to CMS), CCI-control (CCI animals not subjected to CMS), and CCI-CMS (CCI animals subjected to CMS). All the behavioral tests were performed during the light diurnal cycle except the anhedonia test, which was performed in the dark phase. All studies were performed and analyzed in blind conditions.

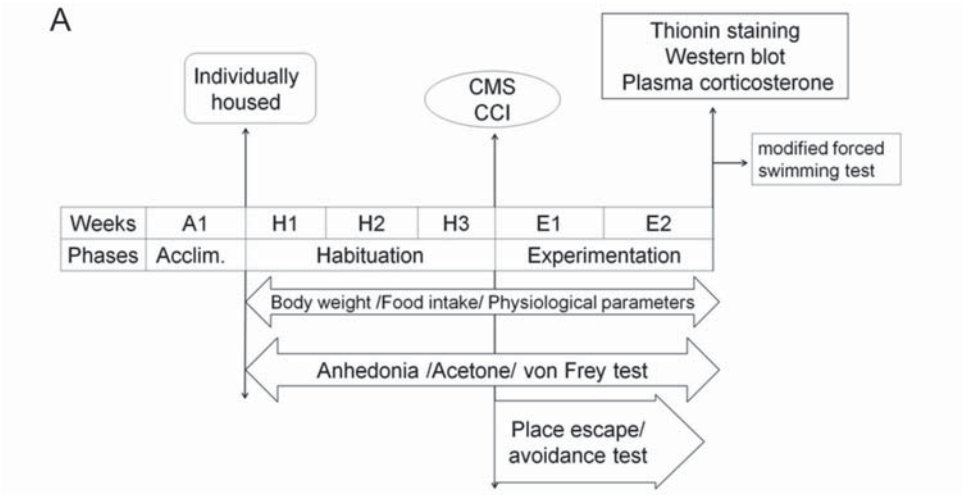
Animal Models

CCI Model of Neuropathic Pain. CCI was produced as described previously.[12,20] Briefly, rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and the left sciatic nerve was then exposed at the mid-thigh level, proximal to the sciatic trifurcation. Four chrome gut (4–0) ligatures were tied loosely around the nerve, separated by 1.0–1.5 mm so as not to compromise the vascular supply. The overlying layers of muscle were closed with 4–0 nonabsorbable silk thread and the skin sutured with 2–0 silk thread. Sham operations were performed in the same manner but without nerve ligation. Animals that had postsurgical complications or exhibited autotomy of at least one distal phalange of the operated paw were excluded from the study.

CMS Model of Depression. CMS was performed as described previously by Willner with minor modifications.[18] This model involved exposure to a series of different insults that were changed daily, using a weekly rotation plan for a period of 14 days (fig. 1B). The stresses included: food deprivation, water deprivation, cage tilting (45 degrees), soiled cage, group housing after a period of water deprivation, stroboscopic illumination (130 flashes/min), and intermittent illumination every 2 h. Control rats (nonstressed) were housed under similar conditions but they were not disturbed during the period of stress.

Assessment of Sensory Pain

Cold Allodynia (Acetone Test). To investigate sensory pain processing, cold allodynia was evaluated once a week during habituation (H3) and experimentation phases (fig. 1A). Animals were placed on an increased wire mesh floor and con-



B

Chronic mild stress

Time (h)	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
08:30-18:30	Water deprivation	von Frey/ acetone test	Grouped housing	Water deprivation/ Food deprivation	Place escape/ avoidance test	No stress	No stress
18:30-08:30	Intermittent illumination	Water deprivation	Cage tilting	Anhedonia test	Stroboscopic illumination	Intermittent illumination	Physiological parameters/ Food deprivation

Fig. 1. Schematic representation of (*A*) the experimental design and (*B*) stressors and tests used in the CMS procedure. Acclim (A1) = acclimatization phase; CCI = chronic constriction injury; CMS = chronic mild stress; E1–E2 = first and second week of the experimentation phase; H1–H3 = first, second, and third week of the habituation.

fined individually in polymethyl methacrylate boxes (18.5 × 21 × 13.5 cm). The rats were allowed to adapt to the testing environment for at least 15 min before a drop of acetone (100 μl) was applied to the center surface of the hind paw with a pipette. Acetone was applied alternately five times to each hind paw, at 5- min intervals and the responses were recorded over a 1-min period after application as follows according to the scale described previously:[21] 0, no response; 1, quick withdrawal, flick or stamp of the paw; 2, prolonged withdrawal or repeated flicking of the paw; 3, repeated flicking of the paw with persistent licking directed at the ventral side of the paw. The cumulative scores were obtained by summing the scores for each rat and dividing by 5, the number of assays.

Mechanical Allodynia (von Frey Test). To study mechanical sensory pain, the mechanical threshold (expressed in grams) was measured in rats using an automatic von Frey apparatus (Dynamic Plantar Aesthesiometer, Ugo Basile, Comerio, Italy).[22] The animals were placed on the same chambers used for the acetone test and habituated to their environment for 30 min. A vertical force was then applied to the left hind paw increasing from 0 to 50 g over a period of 20 s, and the threshold was determined as the force that induced a withdrawal response, with a 50-g cutoff limit. Paw withdrawal thresholds were determined once a week during habituation (H3) and experimentation phases (fig. 1A).

Assessment of Affective Pain: Place Escape/Avoidance Test

This test was performed in a quiet and light-attenuated room divided into two compartments (2.38 × 2.93 × 3.00 m each), one for habituation and the other for testing. Rats were brought into the habituation room at least 30 h before the test session to habituate them to the environment. The test was performed in a standard place-conditioning apparatus once a week during the experimentation phase [15] (fig. 1A). Animals were placed on the center of an increased metal grid (60 × 30 × 30 cm). One half of the chamber was white (bright/anxiogenic area) and the other half black (dark/non-anxiogenic area). Once in the chamber, the rats were allowed to move freely in the chamber and during the ensuing 30-min test period, the animals were stimulated mechanically at 15-s intervals on the plantar surface of the hind paw with a von Frey monofilament (60 g), depending on their location

in the box. Thus, if the animals were in the dark side of the chamber the injured hind paw (ipsilateral) was stimulated, whereas the noninjured hind paw (contralateral) was stimulated when the animals were in the bright side. Hence, animals were obliged to "choose" what they considered to be the less aversive side of the chamber: a dark-nonanxiogenic place in which the injured paw was stimulated or a bright mildly anxiogenic place in which the noninjured paw is stimulated. Every test session was recorded and subsequently analyzed with Spontaneous Motor Activity Recording and Tracking software (Panlab S.L.U., Barcelona, Spain). The percentage of time spent in the light area was scored at 5-min time intervals. Additional parameters indicative of spontaneous motor activity in the light and dark areas were also evaluated: percentage of distance traveled in each area, mean velocity, percentage of resting time, percentage of moving slow, and percentage of moving fast. The latency of paw withdrawal in response to mechanical von Frey stimulation (60 g) was also evaluated to identify differences in the intensity of pain stimulation (sensorial pain) across the experimental groups.

Assessment of Depressive-like Behaviors

Anhedonia Test. One of the core symptoms of clinical depression is the loss of interest in normally rewarding stimuli: anhedonia, which was assessed as described previously with only minor modifications.[23] The consumption of sweet cereals (Kellogg's All Bran® fruit snacks (Kellogg's, Barcelona, Spain): fig. 1A) was tested after 10 h of food and water deprivation once a week during habituation and experimentation phases (fig. 1A). Thirty grams of sweet cereals were placed for 14 h in one extremity of the animal box and their consumption was estimated simultaneously in each group by comparing what remained at the end of the test. Animals received again food and water *ad libitum* after the test. Cereal intake was expressed as g cereal · kg^{-1} body weight.

Modified Forced Swimming Test. The modified forced swimming test was used to evaluate behavioral despair in one set of animals at the end of the experimentation period. Rats were placed individually into polymethyl methacrylate cylinders (height 40 cm, diameter 18 cm) filled with tap water (25 ± 1°C, 30 cm deep).[24] A 15-min pretest was initially performed, followed by a 5 min test 24 h later. Climbing was defined as upward-directed movements of the forepaws along the side of the swim chamber. Swimming was defined as active swimming in the chamber and crossing into another quadrant. Immobility was defined as a lack of activity other than the movements necessary to keep the rat's head above the water. Depressive-like behavior (behavioral despair) was defined as an increase in the time (in seconds) spent immobile. As a positive control, the antidepressant desipramine (20 mg/kg; Sigma–Aldrich, St Louis, MO) was administered intraperitoneal to naïve rats 23.5, 5, and 1 h before testing. Swimming and climbing behaviors have been correlated with increases in serotonergic and catecholaminergic neurotransmission, respectively.

Behavioral Physiologic Parameters

Body weight and food intake were measured once a week during both the habituation and experimentation phases (fig. 1). In addition, the state of the fur was measured once a week using a scale from 1 to 3: a healthy state was scored as 3; a damaged state with piloerection and/or dirty fur was scored as 1; and an intermediate state was scored as 2.[25,26]

Plasma Corticosterone

A separate set of animals was used to measure plasma corticosterone. Animals were sacrificed by administration of chloral hydrate (400 mg/kg intraperitoneal) and blood was collected by cardiac puncture in the presence of trisodium citrate to avoid coagulation (3.15% in phosphate buffered saline: 1 vol citrate per 9 vol blood). Blood was collected the day after the last CMS session, between 9:00 AM and 11:00 AM, using a procedure that takes no longer than 3 min (from anesthesia to blood extraction). After centrifugation at 1,000 g for 15 min, the plasma was collected and stored at −20°C before it was assayed using a commercial radioimmunoassay ^{125}I-labeled rat corticosterone kit (Siemens Healthcare Diagnostics, Barcelona, Spain). The detection limit of the kit is 5.7 ng/ml, the intraassay coefficient variation is 4.3%, and the interassay variation is 5.8%. A γ counter (Wallac Wizard 1470, Perkin Elmer, Waltham, MA) was used to measure the radioactivity of the samples.

Anterior Cingulate Cortex Studies

Histochemistry. After 2 weeks of CCI and/or CMS, one set of animals was perfused through the ascending aorta with 250 ml oxygenated Tyrode solution followed by 750 ml 4% paraformaldehyde. The brain was removed and post fixed in the same fixative solution for 2 h, and then cryoprotected (sucrose 30% in phosphate buffer 0.1 M) before obtaining serial cryostat sections (20 μm) of the anterior cingulate cortex (ACC) collected on gelatinized glass slides. Every eighth section (from 3.70 to −1.40 mm to bregma) was processed for thionin staining after washing in H_2O and incubating in acetone acid. The sections were stained in 0.1% thionin in 10% formol solution for 30 s and then cleared in xylene before adding a coverslip. To quantify the neuronal density in the ACC, two fixed areas of 0.25 × 0.25 mm were selected in each section (Olympus BX60, Center Valley, PA) and the number of thionin-stained neurons was recorded and averaged for each animal.

Western Blots. Another set of animals was sacrificed with an overdose of chloral hydrate, the ACC was dissected out and this tissue was stored at −80°C.[27] The frozen tissue was then solubilized in a solution containing 50 mM Tris-hydrochloride (pH 7.7), as well as protease and phosphatase inhibitors. Samples were sonicated and protein concentration was determined by the Bradford method. Fifty μg of protein of each sample were mixed with loading buffer (sodium dodecyl sulfate 4% p/v, glycerol 20% v/v, β-mercaptoethanol 10% v/v, EDTA 25 mM, bromophenol blue 0.08%) and were

resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (10–20%) and transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA). Subsequently, the membrane was blocked with 5% of bovine serum albumin in Tris buffer solution plus 1% Tween 20 during 1 h and incubated with primary antibodies against phosphorylated isoforms of extracellular signal-regulated kinases 1 and 2 (p-ERK rabbit; dilution 1:5000, Acris Antibodies, Herford, Germany) and mouse antitotal-ERK1/2 (t-ERK; 1:2000, Cell Signaling Technology, Danvers, MA) at 4°C overnight. The membranes were washed in Tris buffer solution plus 1% Tween 20 and incubated for 1 h at 25°C with the corresponding horseradish peroxidase conjugated secondary antibody (diluted 1:10,000 in Tris buffer solution plus 1% Tween 20). Antibody binding was detected by enhanced chemiluminescence and appropriate film exposures were digitalized with Versadoc 5000 (Bio-Rad) using Quantity-One Software v 4.6.9. (Bio-Rad), and then quantified with ImageJ Software (National Institutes of Health, Bethesda, MD). The level of p-ERK and t-ERK were normalized to the level of α-tubulin. All the data are presented as the normalized levels.

Statistical Analysis

All data are presented as the mean + SEM and all the results were analyzed using STATISTICA 10.0 (StatSoft, Tulsa, OK) software, using either an unpaired Student *t* test (two-tailed), or two-way or three-way analysis of variance (ANOVA) with or without repeated measures, as appropriate. The independent variables were CCI (between-groups), CMS (between-groups) and Time (within-groups). To assess if food intake was affecting the evolution of cereal intake, a model using Generalized Estimating Equations[28] was constructed using Statistical Package for the Social Sciences 18 (IBM, New York, NY) software. The dependent variable was Cereal Intake and the independent variables were Time and Food Intake, this latter variable entered as a time-varying covariate. The level of significance was considered as $P < 0.05$.

Results

Effect of Chronic Pain and Depression on Sensory Pain

The effects of chronic pain and CMS on cold allodynia were evaluated using the acetone test (fig. 2). A three-way repeated measures ANOVA for the ipsilateral hind paw revealed a significant Time x CCI x CMS interaction ($P = 0.029$: table 1). Subsequent analysis indicated that animals subjected to pain (in both the CCI-control and CCI-CMS groups) exhibited similar severe cold allodynia of the ipsilateral hind paw 1 and 2 weeks postsurgery, when compared with sham-control or sham-CMS groups (fig. 2A). Cold allodynia in the ipsilateral hind paw was also observed in the sham-CMS group ($P = 0.007$ and P less than 0.0001, respectively: fig. 2A), although this allodynic effect was less intense than that observed in CCI-control or CCI-CMS animals. An analysis of

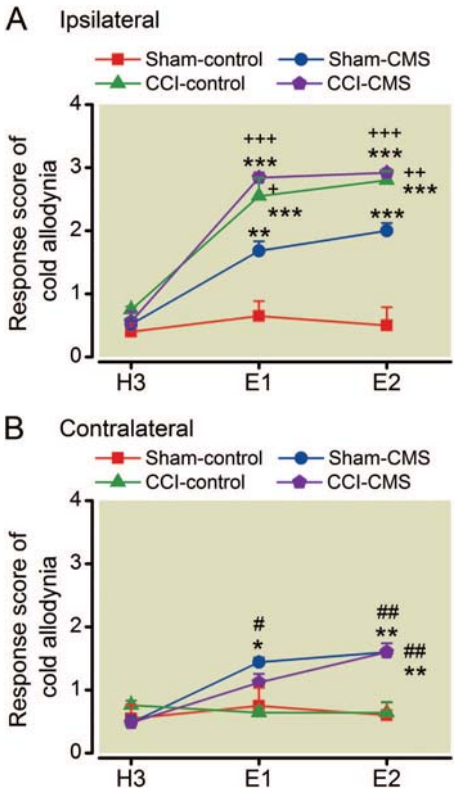


Fig. 2. Effect of chronic neuropathic pain and chronic mild stress in the acetone model; (*A*) ipsilateral and (*B*) contralateral hind paw. Experimental groups: Sham-control (sham-operated without CMS, n = 10), Sham-CMS (sham-operated subjected to CMS, n = 10), CCI-control (neuropathic pain animals not subjected to CMS, n = 9), and CCI-CMS (neuropathic pain animals subjected to CMS, n = 10). Each symbol represents the mean + SEM. Statistical analysis at each time period: **P* less than 0.05, ***P* less than 0.01, ****P* less than 0.001 *versus* sham-control; +*P* less than 0.05, ++*P* less than 0.01, +++*P* less than 0.001 *versus* sham-CMS; #*P* less than 0.05, ##*P* less than 0.01 *versus* CCI-control. CCI = chronic constriction injury; CMS = chronic mild stress; E1 and E2 = first and second week of the experimentation phase; H3 = third week of the habituation.

the data from contralateral paw also revealed a significant interaction between Time x CMS (*P* less than 0.0001: table 1). In agreement with data from the contralateral paw, both CMS groups (sham-CMS and CCI-CMS) exhibited cold allodynia when compared with sham-controls in the second week of experimentation phase ($P = 0.004$: fig. 2B). As expected, no effects were observed in the contralateral paw of the CCI-control animals.

The effects of chronic pain and CMS on the mechanical sensory component of pain were evaluated using the von Frey test (fig. 3). A three-way repeated measures ANOVA of the ipsilateral hind paw revealed a statistically significant effect for the Time*CCI factor (*P* less than 0.0001: table 1) but no significant effect of CMS ($P = 0.814$). Severe mechanical allodynia of the ipsilateral hind paw was evident in the CCI-control and CCI-CMS groups when they were compared

Table 1. Summary of the Statistical Analyses

Behavioral Tests	CCI	CMS	CCI*CMS	Time	Time*CCI	Time*CMS	Time* CCI*CMS
				Acetone test			
I	$F_{(1.35)} = 98.89^{***}$	$F_{(1.35)} = 18.31^{*}$	$F_{(1.35)} = 13.16^{**}$	$F_{(2.70)} = 153.03^{***}$	$F_{(2.70)} = 35.02^{***}$	$F_{(2.70)} = 11.26^{***}$	$F_{(2.70)} = 4.01^{*}$
C	$F_{(1.35)} = 0.078$	$F_{(1.35)} = 18.63^{*}$	$F_{(1.35)} = 0.51$	$F_{(2.70)} = 20.66^{***}$	$F_{(2.70)} = 1.75$	$F_{(2.70)} = 21.99^{***}$	$F_{(2.70)} = 0.15$
				von Frey test			
I	$F_{(1.36)} = 97.40^{***}$	$F_{(1.36)} = 0.01$	$F_{(1.36)} = 0.09$	$F_{(2.72)} = 42.46^{***}$	$F_{(2.72)} = 47.62^{***}$	$F_{(2.72)} = 1.05$	$F_{(2.72)} = 0.42$
C	$F_{(1.36)} = 0.01$	$F_{(1.36)} = 1.36$	$F_{(1.36)} = 1.33$	$F_{(2.72)} = 0.78$	$F_{(2.72)} = 0.22$	$F_{(2.72)} = 0.85$	$F_{(2.72)} = 0.87$
				Place escape/avoidance test			
1st week	$F_{(1.34)} = 7.30^{*}$	$F_{(1.34)} = 0.93$	$F_{(1.34)} = 2.37$	$F_{(5.170)} = 1.47$	$F_{(5.170)} = 3.74^{**}$	$F_{(5.170)} = 0.19$	$F_{(5.170)} = 1.46$
2nd week	$F_{(1.34)} = 53.55^{***}$	$F_{(1.34)} = 9.77^{**}$	$F_{(1.34)} = 7.52^{*}$	$F_{(5.170)} = 1.74$	$F_{(5.170)} = 13.11^{***}$	$F_{(5.170)} = 0.81$	$F_{(5.170)} = 1.26$
Anhedonia	$F_{(1.41)} = 0.05$	$F_{(1.41)} = 14.29^{***}$	$F_{(1.41)} = 0.196$	$F_{(4.164)} = 16.08^{***}$	$F_{(4.164)} = 0.36$	$F_{(4.164)} = 16.63^{***}$	$F_{(4.164)} = 0.92$
				Modified forced swimming test			
Immob.	$F_{(1.35)} = 0.00$	$F_{(1.35)} = 16.44^{***}$	$F_{(1.35)} = 1.14$	—	—	—	—
Swimming	$F_{(1.35)} = 0.86$	$F_{(1.35)} = 0.02$	$F_{(1.35)} = 0.00$	—	—	—	—
Climbing	$F_{(1.35)} = 0.11$	$F_{(1.35)} = 14.82^{***}$	$F_{(1.35)} = 1.04$	—	—	—	—
				ACC			
Thionin	$F_{(1.36)} = 20.59^{***}$	$F_{(1.36)} = 4.72^{*}$	$F_{(1.36)} = 0.97$	—	—	—	—
p-ERK	$F_{(1.12)} = 10.77^{**}$	$F_{(1.12)} = 18.95^{***}$	$F_{(1.12)} = 0.00$	—	—	—	—
t-ERK	$F_{(1.9)} = 0.00$	$F_{(1.9)} = 2.35$	$F_{(1.9)} = 1.91$	—	—	—	—

Data were analyzed by two-way or three-way analysis of variance (ANOVA), with or without repeated measures, as appropriate. The independent variables were CCI (between-groups), CMS (between-groups) and Time (within-groups). Significant results are indicated in bold.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

ACC = anterior cingulate cortex; C = contralateral; CCI = chronic constriction injury; CMS = chronic mild stress; I = ipsilateral; Immob = immobility; p-ERK = phosphorylated extracellular signal-regulated kinases 1 and 2; t-ERK = total extracellular signal-regulated kinases 1 and 2.

with the sham-control or sham-CMS animals at 2 weeks postsurgery (*P* less than 0.0001: fig. 3A). No differences were found between the CCI-control and CCI-CMS groups (fig. 3A). In the contralateral hind paw no alterations in the mechanical pain threshold were evident in any of the groups of rats (table 1 and fig. 3B). Overall, the sensorial responses of the ipsilateral hind paw to mechanical stimulation were similar in both CCI groups (CCI-control and CCI-CMS), and no decrease in the mechanical pain threshold (ipsilateral or contralateral) was observed in the sham-CMS group.

Effect of Chronic Pain and Depression on Affective Pain

First, we explored the latency of paw withdrawal in response to a hyperalgesic von Frey monofilament (60 g) in the experimental groups, confirming that the withdrawal latency to this noxious stimulus was similar between the CCI-control and CCI-CMS groups, and between sham-control and sham-CMS animals during the experimentation phase (data analyzed using three-way ANOVA, not shown). As the CCI-control and CCI-CMS groups both showed similar sensorial responses to mechanical pain, we evaluated the affective dimension of pain in the place escape/avoidance test using a von Frey monofilament (60 g). In this test after 1 week, a significant effect of CCI and the CCI x Time interaction was evident on the time spent in the white area when analyzed with a three-way repeated measures ANOVA ($P = 0.011$ and $P = 0.003$, respectively: table 1). Subsequent *post hoc* tests revealed an increase in the time spent within the bright area at 25 and 30 min in the CCI-CMS *versus* the sham-control group ($P = 0.032$ and $P = 0.031$, respectively: fig. 4A). In agreement with previous data,[15] CCI-control animals also showed a statistically significant increase in this parameter at 30 min ($P = 0.030$: fig. 4A). These effects were greater after 2 weeks. Three-way repeated measures ANOVA (table 1) demonstrated statistically significant interactions of CCI x CMS and Time x CCI ($P = 0.010$ and *P* less than 0.0001, respectively; table 1). A statistically significant increase in the time spent in the bright area was observed in CCI-CMS and CCI-control groups when compared with sham-controls from 10 min and 15 min, respectively (fig. 4B). Importantly, the CCI-CMS group escaped more from the dark side than the CCI-controls at 20, 25 and 30 min ($P = 0.014$, $P = 0.038$ and $P = 0.015$, respectively: fig. 4B). Sham-CMS animals with no paw injury and that exhibited no changes in sensitivity to mechanical pain had similar behavioral profiles to sham-control animals at both time points. We evaluated several parameters of motor activity (distance traveled in each area, mean velocity, resting time, slow and fast movement) to rule out the possibility that motor impairment was masking the effects in any group, demonstrating that there were no motor differences between groups (data analyzed using two-way ANOVA, not shown). Taken together, these findings suggest that the induction of a depressive-like state altered the affective-emotional component of pain in animals suffering chronic pain.

Effect of Chronic Pain and Depression on Depressive-like Behaviors

To evaluate the influence of chronic pain in classic depression paradigms, we studied the development of anhedonia. As expected, there was a significant Time x CMS interaction (*P* less than 0.0001: table 1), but no significant effect of CCI

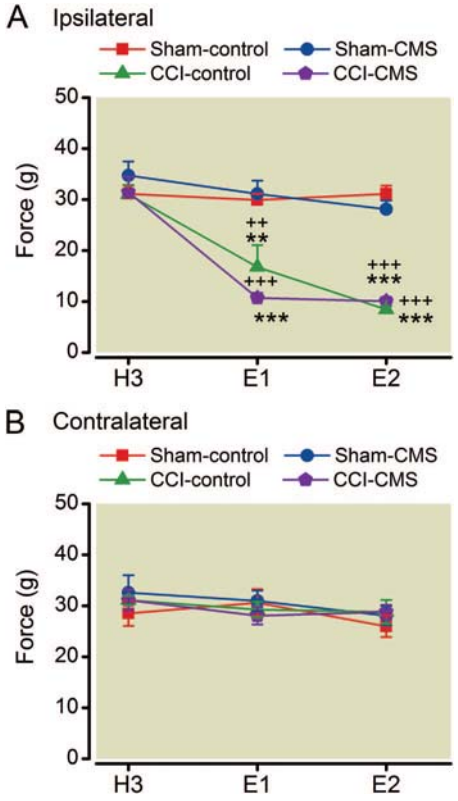


Fig. 3. Effect of chronic neuropathic pain and chronic mild stress on (*A*) ipsilateral and (*B*) contralateral hind paw responses in the von Frey test. Experimental groups: Sham-control (sham-operated without CMS, n = 11), Sham-CMS (sham-operated subjected to CMS, n = 8), CCI-control (neuropathic pain animals not subjected CMS, n = 10) and CCI-CMS (neuropathic pain animals subjected to CMS, n = 11). Each symbol represents the mean + SEM. Statistical analysis at each time period: ***P* less than 0.01, ****P* less than 0.001 *versus* sham-control; ++*P* less than 0.01, +++*P* less than 0.001 *versus* sham-CMS. CCI = chronic constriction injury; CMS = chronic mild stress; E1 and E2 =first and second week of the experimentation phase; H3 = third week of the habituation.

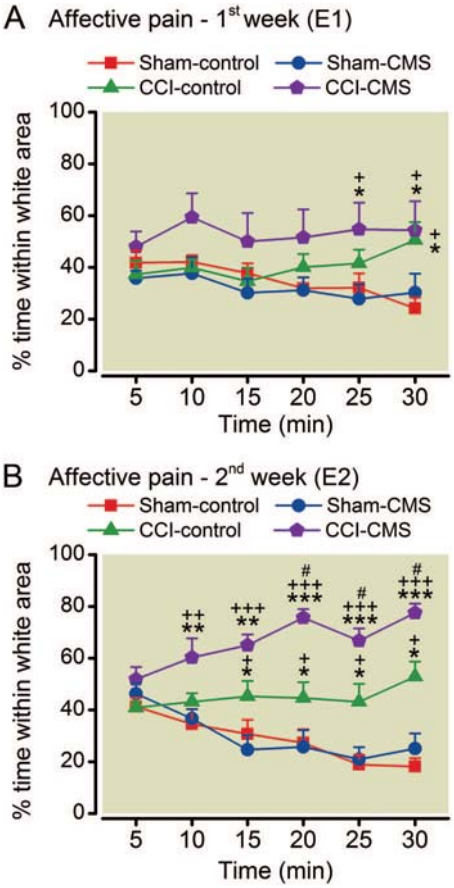


Fig. 4. Effect of chronic neuropathic pain and chronic mild stress in the place escape/avoidance test. Percentage time spent within the white area (*A*) 1 week and (*B*) 2 weeks after beginning the CMS and CCI protocols. Experimental groups: Sham-control (sham-operated without CMS, n = 10), Sham-CMS (sham-operated subjected to CMS, n = 10), CCI-control (neuropathic pain animals not subjected CMS, n = 10) and CCI-CMS (neuropathic pain animals subjected to CMS, n = 8). Each symbol represents the mean + SEM. Statistical analysis at each 5-min time period: **P* less than 0.05, ***P* less than 0.01, ****P* less than 0.001 *versus* sham-control; +*P* less than 0.05, ++*P* less than 0.01, +++*P* less than 0.001 *versus* sham-CMS; #*P* less than 0.05 *versus* CCI-control. CCI = chronic constriction injury; CMS = chronic mild stress; E1 and E2 = first and second week of the experimentation phase.

(*P* = 0.956) was observed. Accordingly, groups subjected to the depression paradigm (sham-CMS and CCI-CMS) exhibited statistically significant anhedonia after 1 and 2 weeks when compared with CCI-control or sham-control groups (both *P* less than 0.0001: fig. 5A). Interestingly, similar levels of anhedonia were found in both CMS groups whereas CCI-control animals exhibited a similar behavioral profile to the sham-control group.

We also studied behavioral despair using the modified forced swimming test (fig. 5B) 14 days after inducing CCI and CMS. Two-way ANOVA revealed a significant effect of CMS on immobility and climbing behaviors (*P* less than 0.0001: table 1), with a statistically significant increase in the immobility time in sham-CMS and CCI-CMS animals (fig. 5B). This effect may well be related to impaired catecholaminergic neurotransmission given the significant decrease in climbing behavior in both groups (*P* = 0.005 and *P* = 0.021, respectively *vs.* sham-control: fig. 5B). There was no difference in the time the animals in the different groups spent swimming. Together, these findings demonstrate that CMS produced similar depressive-like behaviors in sham-CMS and CCI-CMS animals, responses that, in agreement with our anhedonia data, were not induced in CCI-control animals. Finally and as a measure of the validity of the test, desipramine administration significantly decreased the immobility time (unpaired Student *t* test (two-tailed), *P* = 0.004) while it increased the climbing time when compared with the sham-control animals (unpaired Student *t* test (two-tailed), *P* = 0.006: fig. 5B).

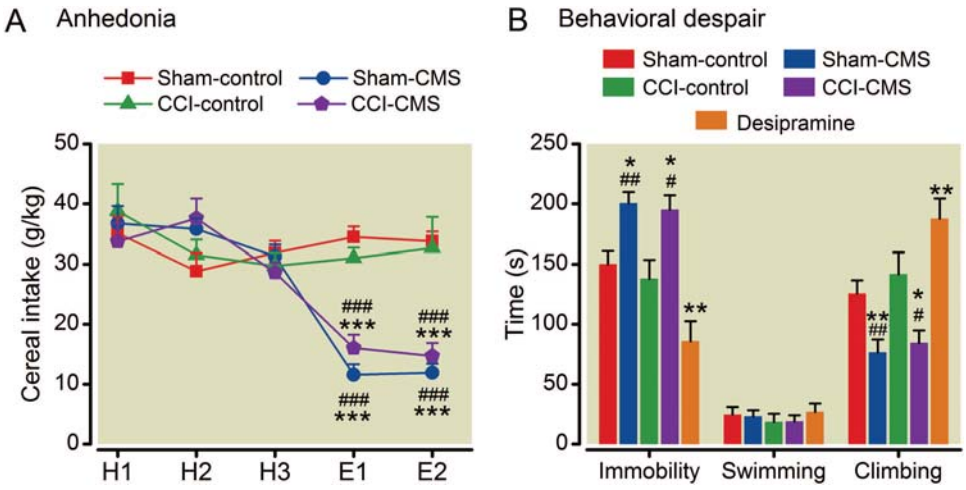


Fig. 5. Effect of chronic neuropathic pain and chronic mild stress on depressive-like behaviors. (*A*) Anhedonia was evaluated by measuring the intake of sweet cereals (g cereal · kg^{-1} body weight), 1 week and 2 weeks after beginning the CMS and CCI protocols. Experimental groups: Sham-control (sham-operated without CMS, n = 10); Sham-CMS (sham-operated subjected to CMS, n = 11); CCI-control (neuropathic pain animals not subjected to CMS, n = 12); CCI-CMS (neuropathic pain animals subjected to CMS, n = 12). (*B*) Behavioral despair was evaluated in the modified forced swimming test after 2 weeks of CMS and CCI. Experimental groups: Sham-control (n = 10); Sham-CMS (n = 10); CCI-control (n = 9); CCI-CMS (n = 10); and Desipramine (DMI) (naïve animals treated with desipramine 20 mg/kg, intraperitoneal, 23.5, 5, and 1 h before the test, n = 7). Each symbol represents the mean + SEM. Statistical analysis: **P* less than 0.05, ***P* less than 0.01, ****P* less than 0.001 *versus* sham-control; #*P* less than 0.05, ##*P* less than 0.01, ###*P* less than 0.001 *versus* CCI-control. CCI = chronic constriction injury; CMS = chronic mild stress; E1 and E2 = first and second week of the experimentation phase; H1, H2, and H3 = first, second, and third week of habituation.

Effect of Chronic Pain and Depression on Physiologic Behavioral Parameters

All CMS groups (sham-CMS and CCI-CMS) suffered a decrease in body weight and food intake, as well as a statistically significant deterioration in their physical state at the end of the experimentation phase (fig. 6). Next, we evaluated if the development of anhedonia was affected by food intake. The Generalized Estimating Equations model showed that food intake did not have a significant effect on the evolution of cereal intake (B = 0.13; 95% confidence interval = 0.78, 1.67, $P = 0.515$).

Effect of Chronic Pain and Depression on Plasma Corticosterone Levels

There were no statistically significant differences in plasma corticosterone in either sham-CMS (132.08 ± 29.49 ng/ml, n = 12), CCI-control (203.34 ± 18.47 ng/ml, n = 12), or CCI-CMS (163.07 ± 21.53 ng/ml, n = 12) when compared with the sham-control animals (193.33 ± 23.27 ng/ml, n = 14) at the end of the experimentation phase.

Effect of Chronic Pain and Depression in the ACC

Because it has been suggested that the ACC is implicated in the processing of emotions in both pain and depression, we explored the neuronal density and ERK activity in the ACC to determine whether the coexistence of both modifies these parameters (fig. 7 A-H). Two-way ANOVA revealed a significant effect of both CCI and CMS on thionin staining (*P* less than 0.0001 and $P = 0.036$, respectively; table 1). The density of the stained neurons decreased, although not statistically significant, in the CCI-control *versus* sham-control animals ($P = 0.08$). However, a statistically significant decrease in thionin-stained neurons was observed in the CCI-CMS group when compared with sham-control and sham-CMS groups (*P* less than 0.0001 and $P = 0.008$, respectively: fig. 7A), indicating a loss of neuronal bodies in the ACC of CCI-CMS animals. In addition, ERK activity in the ACC was also explored (fig. 7 F-H). A two-way ANOVA analysis showed a significant effect of CMS and CCI on this parameter (*P* less than 0.0001 and $P = 0.006$, respectively: table 1), and the subsequent *post hoc* test revealed that the twofold increase in the amount of p-ERK in CCI-control and sham-CMS animals when compared with the sham-control rats was not statistically significant. However, there was a significant increase in the accumulation of p-ERK in the CCI-CMS group compared with the sham-control rats (*P* less than 0.0001: fig. 7F-G) that was not accompanied by changes in t-ERK expression (fig. 7H and table 1).

Discussion

To our knowledge, this is the first study to assess the effects of comorbid chronic pain and depression in an animal model, addressing both the somatic and emotional components of each condition. We demonstrate that the induction of an experimental depressive-like state negatively affects the affective component of chronic pain, while decreasing neuronal density and increasing ERK activation in the ACC. The depressive-like state also exerted a negative effect on the sensory

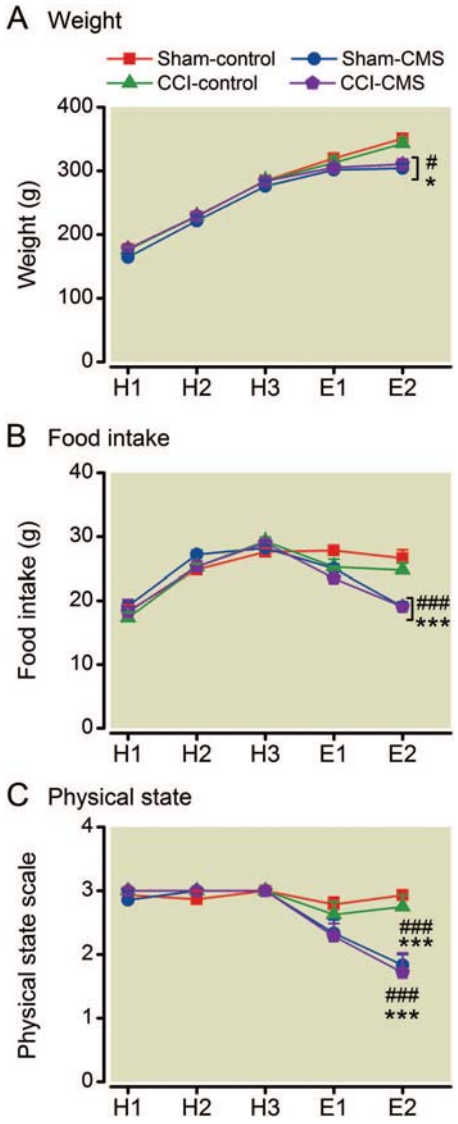


Fig. 6. Effect of chronic neuropathic pain and chronic mild stress on (*A*) weight, (*B*) food intake, and (*C*) physical state over time. Experimental groups: Sham-control (sham-operated without CMS, n = 10), Sham-CMS (sham-operated subjected to CMS, n = 11), CCI-control (animals with neuropathic pain not subjected CMS, n = 12), and CCI-CMS (animals with neuropathic pain subjected to CMS, n = 12). Each symbol represents the mean + SEM. Statistical analysis: **P* less than 0.05, ****P* less than 0.001 *versus* sham-control; #*P* less than 0.05, ###*P* less than 0.001 *versus* CCI-control. CCI = chronic constriction injury; CMS = chronic mild stress; E1 and E2 = first and second week of the experimentation phase; H1, H2, and H3 =first, second and third week of the habituation.

component of pain, although only for specific pain modalities (summary in table 2).

Using withdrawal reaction tests, we first explored the effect of CMS on sensory pain, both alone and in combination with chronic pain. As expected, the induction of peripheral neuropathic pain caused mechanical and cold allodynia in the ipsilateral hind paw, which was unaffected by the induction of a depressive-like state. CMS animals exhibited cold allodynia in both hind paws, which was no different in the animals also subjected to CCI. Thus, in accordance with data both from patients and laboratory animals, the induction of a depressive-like state appears to provoke aberrant or maladaptive central pain processing, which affects specific stimulus modalities in our controlled experimental conditions, such as cold stimulus in this case. Hypoalgesia to thermal or electrical stimuli[9,11,29] and decreased nociceptive thresholds to experimentally evoked pain[30] have been reported in depressed patients. Moreover, rats subjected to CMS showed increased nociceptive tail-flick test thresholds in preclinical studies,[31] whereas chronic restraint stress over 1 week induced mechanical and cold allodynia.[32] Although these findings do not demonstrate a direct link between depression and global alterations to the sensory dimension of pain, they indicate that the nature of the sensorial nociceptive input has a substantial effect on the development of pain disorder in depression.

The main finding of the current study is that aversion to a painful experience is selectively increased by a coexisting depressive state. By contrast, chronic pain had no effect on the characteristic depressive-like profile of animals subjected to CMS. Studying the affective component of pain in rodents has previously proved to be a challenging task, although this field of research has been greatly aided by the development of the place escape/avoidance test.[15,33] This test permits the aversive nature of a noxious stimulus to be assessed based on the avoidance of a preferred location where the stimulus is delivered. Accordingly, the CCI-control group showed a higher preference for the bright area than the sham-control and sham-CMS groups. Strikingly, however, this preference was greater in animals suffering a depression-like state and concomitant chronic pain, suggesting that this group has the most negative pain experience, in agreement with human studies.[34–36] Although these studies had short-lasting effects and they were performed mostly in healthy volunteers, the results reinforce the idea that persistent mood changes may lead to a more negative interpretation of painful experiences. To support the validity of the model used, we selected a nociceptive input that caused a similar withdrawal reaction in CCI-control and CCI-CMS rats. Effective discrimination between the sensorial and emotional effect of analgesic drugs has already been demonstrated using this paradigm.[33] Taken together with our observations, these findings suggest that comorbidity of depression and pain leads to a more negative perception of noxious stimuli than when pain is experienced without depression.

Importantly, we found that changes in the perception of affective pain in CCI-CMS rats were not accompanied by a worsening of the depressive-like state. Indeed, rats subjected to CMS with or without chronic pain displayed similar levels of anhedonia, a core symptom of depression.[18] In addition, both groups displayed similar levels of behavioral despair in the modified forced swimming test, as determined by an

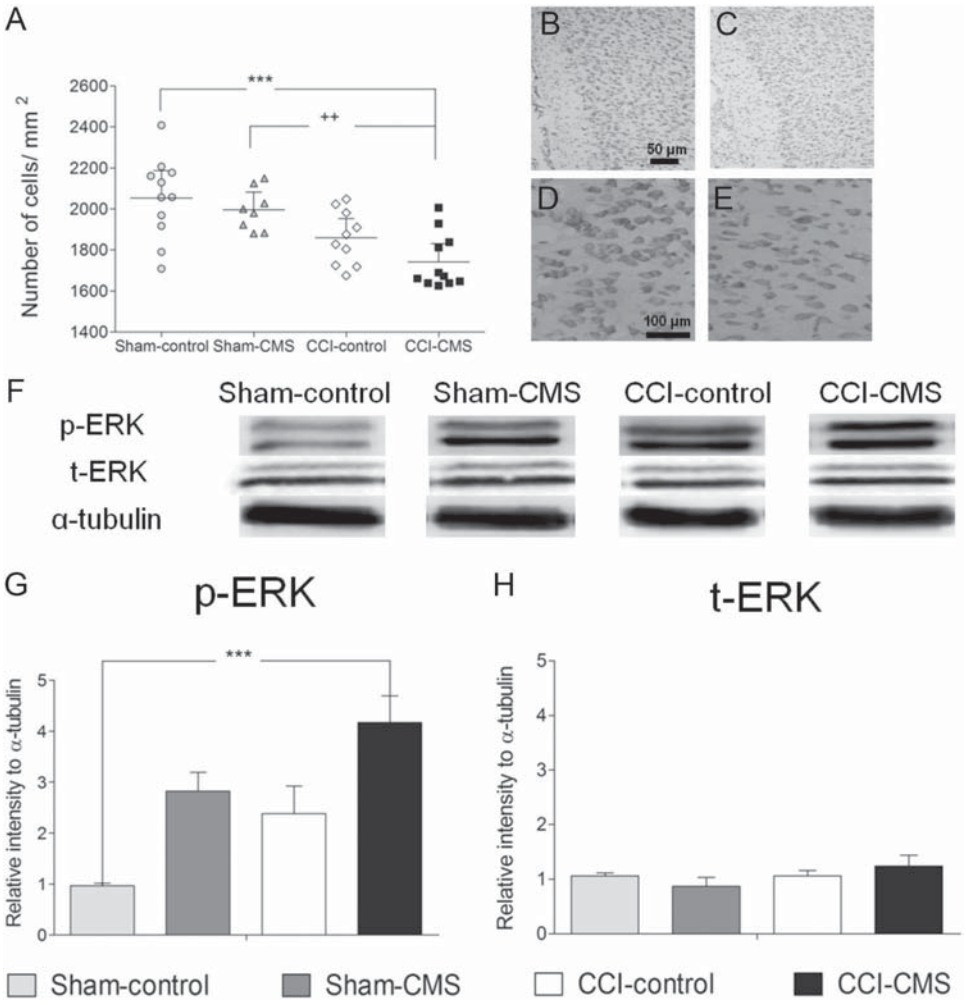


Fig. 7. Neuronal density (*A-E*) and extracellular signal-regulated kinases 1 and 2 (ERK) activity in the anterior cingulate cortex (*F–H*) after 2 weeks of chronic neuropathic pain and chronic mild stress. (*A*) CCI-CMS significantly decreased neuronal density in the anterior cingulate cortex (ACC) (number of neurons counted per visual field). Experimental groups: Sham-control (sham-operated without CMS, n = 11), Sham-CMS (sham-operated subjected to CMS, n = 8), CCI-control (neuropathic pain animals not subjected to CMS, n = 10), and CCI-CMS (neuropathic pain animals subjected to CMS, n = 11). (*B–E*) Photomicrographs of thionin-stained neurons in sham-control (*B*, *D*) and CCI-CMS animals (*C*, *E*). (*F*) Representative Western blot to show the accumulation of activated ERK (p-ERK) and total ERK (t-ERK) in the ACC. (*G–H*) Quantitative analysis of p-ERK (G) and t-ERK (H) relative to α-tubulin. Data represent the mean + SEM of three to four assays performed on ACC samples obtained from six rats per group, where: ****P* less than 0.001 *versus* sham-control; ++*P* less than 0.01, *versus* sham-CMS. CCI = chronic constriction injury; CMS = chronic mild stress.

increase in immobility time that models the psychologic concept of "entrapment." This increase in immobility time was paralleled by a decrease in climbing time, suggesting that the catecholaminergic system is deregulated by depression.[37] Furthermore, some parallels between human depression and chronically stressed animals have been drawn from the reduction in the efficiency with which even the smallest tasks are performed by depressed patients, leading to their inability to maintain minimal personal hygiene, which is mirrored by a decrease in grooming behavior seen in stressed animals (Sham-CMS and CCI-CMS). In addition, the decrease in body weight and food intake in rodents may mimic the weight loss when not dieting or modifications in appetite experienced by depressed patients.[5] By contrast, CCI did not result in depressive-like behavior over 2 weeks, as evident in the anhedonia test, modified forced swimming test, physical state evaluation, food intake, and body weight. This result contradicts the idea that chronic pain can lead to the development of depressive-like and anxiogenic-like behaviors, the so-called "secondary pain affect."[4,38,39] Nevertheless, it should be noted that this secondary pain affect is observed, in our experimental conditions, after 1 month of neuropathic induction.[40] Indeed, a study using spinal nerve ligation in rats found no differences in anxiety and depressive-like behaviors when compared with sham animals 14 days after surgery.[41] Similarly, approximately 30 days of neuropathic pain were necessary to induce a depressive and anxiogenic-like profile in rodents.[38,39,42,43] Overall, these findings show

Table 2. Summary of the Global Effects of Chronic Neuropathic Pain and Chronic Mild Stress, Alone or in Combination

	Sham-control	Sham-CMS	CCI-control	CCI-CMS
Pain				
Sensory pain				
Cold allodynia				
Ipsilateral	0	++	+++	+++
Contralateral	0	++	0	++
Mechanical allodynia				
Ipsilateral	0	0	+++	+++
Contralateral	0	0	0	0
Affective pain				
First week	0	0	+	++
Second week	0	0	++	+++
Depression				
Anhedonia	0	+++	0	+++
Behavioral despair				
Immobility	0	++	0	++
Swimming	0	0	0	0
Climbing	0	++	0	++
Decrease of body weight	0	+	0	+
Decrease of food intake	0	+	0	+
Deterioration of the physical state	0	+++	0	+++
Disruption of the hypothalamic-pituitary-adrenal axis	0	0	0	0
ACC				
Loss of cell density	0	0	0/+	+++
p-ERK activity	0	0/+	0/+	+++

Global effect *vs.* Sham-control group: 0, no effect; +, slight effect; ++, medium effect; +++, strong effect. Experimental groups: Sham-control (sham-operated without CMS), Sham-CMS (sham-operated subjected to CMS), CCI-control (neuropathic pain animals not subjected to CMS) and CCI-CMS (neuropathic pain animals subjected to CMS).

ACC =anterior cingulate cortex; CCI = chronic constriction injury; CMS = chronic mild stress; p-ERK = phosphorylated extracellular signal-regulated kinases 1 and 2.

that comorbid pain and depression first result in emotional impairment related with the perception of pain, after which chronic pain induces secondary pain affects that involve alterations in general mood (depression, anxiety).

The ACC has been proposed to be one of the main areas mediating the relationship between pain and depression, because it is implicated in many activities related to emotional processing, ranging from conflict resolution to anhedonia and negative emotionality.[44] Accordingly, malfunctioning of the ACC has been reported in depression.[45] In the literature related to pain, the ACC been widely implicated in the unpleasantness of noxious stimuli.[46] In our study we found that the neuronal density in the ACC diminished in CCI-CMS animals, consistent with our place escape/avoidance data. The decrease in this neuronal population may be associated with the reduced gray matter in the ACC of patients suffering from chronic pain.[47] Similar findings have been described in the subgenual cortex of patients with major depressive disorder,[48] an area within the ACC thought to be activated only by negative affective experiences.[49] In addition, recent studies further demonstrated that activation of ERKs (members of the mitogen-activated protein kinase family) in the ACC contributes to aversion in response to painful stimuli,[50,51] and tonic increase has been reported in neuropathic pain and chronic stress.[52,53] Accordingly, we found a robust ERK increase in the CCI-CMS rats. Overall, in CCI-CMS animals there is a decrease in neuronal density that is coupled to an increase in ERK activity of the cells remaining in the ACC, which might be related to the behavioral increase of pain-related negative emotion in the CCI-CMS group. Therefore, although no other studies have investigated the effect of comorbid pain and depression, our findings suggest that the coexistence of pain and depression exacerbates the impairments in the ACC. Interestingly, the alterations to the gray matter of the ACC and ERK activation are rescued when pain is effectively controlled.[50,51,54,55] So, it would be of considerable interest to determine whether this recovery is disrupted in cases of comorbidity, which could explain poorer prognosis of these patients.

Although disrupting the hypothalamic-pituitary-adrenal axis has been associated with depression disorders[56] we found no changes in corticosterone levels under our experimental conditions. Increases in corticosterone after CMS have been described,[57] although more recent studies failed to replicate these, in agreement with our results.[58,59] It has been suggested that corticosterone is unaffected due to the adaptive response of hypothalamic-pituitary-adrenal axis to chronic stress. Accordingly, daily exposure to footshock for 14 days induced hypersecretion of corticosterone for the first 7 days, after which its levels returned to control values.[60] Plasma corticosterone levels on the last day of the stress protocol were unchanged from the pretest levels, probably reflecting

adaptation of the hypothalamic-pituitary-adrenal axis as described previously.[61] Although the chloral hydrate was chosen as an anesthetic because it has not been implicated in changes in corticosterone secretion, possible effects of this drug on corticosterone in the specific experimental model used cannot be ruled out. Similarly, no changes in plasma corticosterone levels were detected in CCI animals, consistent with previous studies.[62,63] Together, these findings suggest that hypothalamic-pituitary-adrenal axis dysfunction does not play in major role in the changes described here.

The current study evaluated the relationship between the sensory and emotional dimensions of comorbid pain and depression, by combining two well-validated animal models, the CCI and the CMS, respectively. Our results demonstrate that CMS negatively affects the emotional experience of pain, probably due to ACC impairment, thereby supporting the hypothesis that depression leads to dysfunction in the emotional interpretation of pain in chronic pain conditions. Future animal studies combining distinct models of depression and chronic pain will be vital to elucidate the substrates and mechanisms regulating the sensory and emotional components of pain in cases of comorbidity.

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References

1. Bair MJ, Robinson RL, Eckert GJ, Stang PE, Croghan TW, Kroenke K: Impact of pain on depression treatment response in primary care. Psychosom Med 2004; 66:17–22
2. Karp JF, Scott J, Houck P, Reynolds CF 3rd, Kupfer DJ, Frank E: Pain predicts longer time to remission during treatment of recurrent depression. J Clin Psychiatry 2005; 66:591–7
3. Agera-Ortiz L, Failde I, Mico JA, Cervilla J, Lpez-Ibor JJ: Pain as a symptom of depression: Prevalence and clinical correlates in patients attending psychiatric clinics. J Affect Disord 2011; 130:106–12
4. Price DD: Psychological and neural mechanisms of the affective dimension of pain. Science 2000; 288:1769–72
5. Diagnostic and Statistical Manual of Mental Disorders, Fourth Revision. Washington, D.C., American Psychiatric Press, 2000
6. Bair MJ, Robinson RL, Katon W, Kroenke K: Depression and pain comorbidity: A literature review. Arch Intern Med 2003; 163:2433–45
7. Gureje O: Treating chronic pain in the context of comorbid depression. Pain 2008; 134:3–4
8. Dickens C, McGowan L, Dale S: Impact of depression on experimental pain perception: A systematic review of the literature with meta-analysis. Psychosom Med 2003; 65: 369–75
9. Br KJ, Brehm S, Boettger MK, Wagner G, Boettger S, Sauer H: Decreased sensitivity to experimental pain in adjustment disorder. Eur J Pain 2006; 10:467–71
10. Lautenbacher S, Krieg JC: Pain perception in psychiatric disorders: A review of the literature. J Psychiatr Res 1994; 28:109–22
11. Br KJ, Brehm S, Boettger MK, Boettger S, Wagner G, Sauer H: Pain perception in major depression depends on pain modality. Pain 2005; 117:97–103
12. Bennett GJ, Xie YK: A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988; 33:87–107
13. Kim SH, Chung JM: An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 1992; 50:355–63
14. Johansen JP, Fields HL, Manning BH: The affective component of pain in rodents: Direct evidence for a contribution of the anterior cingulate cortex. Proc Natl Acad Sci U S A 2001; 98:8077–82
15. LaBuda CJ, Fuchs PN: A behavioral test paradigm to measure the aversive quality of inflammatory and neuropathic pain in rats. Exp Neurol 2000; 163:490–4
16. Bessa JM, Mesquita AR, Oliveira M, Pego JM, Cerqueira JJ, Palha JA, Almeida OF, Sousa N: A trans-dimensional approach to the behavioral aspects of depression. Front Behav Neurosci 2009; 3:1
17. Bravo L, Berrocoso E, Mico JA: Animal models in psychiatry: Conceptualization and preclinical models of depression. Eur J Psychiatry 2009; 23:111–22
18. Willner P: Chronic mild stress (CMS) revisited: Consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 2005; 52:90–110
19. Zimmermann M: Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983; 16:109–10
20. Berrocoso E, De Benito MD, Mico JA: Role of serotonin 5-HT1A and opioid receptors in the antiallodynic effect of tramadol in the chronic constriction injury model of neuropathic pain in rats. Psychopharmacology (Berl) 2007; 193:97–105
21. Flatters SJ, Bennett GJ: Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy. Pain 2004; 109:150–61
22. Berrocoso E, Mico JA, Vitton O, Ladure P, Newman-Tancredi A, Depoortre R, Bardin L: Evaluation of milnacipran, in comparison with amitriptyline, on cold and mechanical allodynia in a rat model of neuropathic pain. Eur J Pharmacol 2011; 655:46–51
23. Gamaro GD, Manoli LP, Torres IL, Silveira R, Dalmaz C: Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures. Neurochem Int 2003; 42:107–14
24. Detke MJ, Rickels M, Lucki I: Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology (Berl) 1995; 121:66–72
25. Alonso R, Griebel G, Pavone G, Stemmelin J, Le Fur G, Soubrie P: Blockade of CRF(1) or V(1b) receptors reverses stress-induced suppression of neurogenesis in a mouse model of depression. Mol Psychiatry 2004;9: 278–86, 224
26. Liu Q, Li B, Zhu HY, Wang YQ, Yu J, Wu GC: Glia atrophy in the hippocampus of chronic unpredictable stress-induced depression model rats is reversed by electroacupuncture treatment. J Affect Disord;128: 309–13
27. Paxinos G, Watson C: The Rat Brain in Stereotaxic Coordinates: Compact, 6th Edition. San Diego, CA, Elsevier Academic Press, 2009
28. Dueñas M, Ramirez C, Arana R, Failde I: Gender differences and determinants of health related quality of life in coronary patients: A follow-up study. BMC Cardiovasc Disord 2011; 27:11–24
29. Gormsen L, Ribe AR, Raun P, Rosenberg R, Videbech P, Vestergaard P, Bach FW, Jensen TS: Pain thresholds during and after treatment of severe depression with electroconvulsive therapy. Eur J Pain 2004; 8:487–93

30. Merskey H: The effect of chronic pain upon the response to noxious stimuli by psychiatric patients. J Psychosom Res 1965; 8:405–19
31. Pinto-Ribeiro F, Almeida A, Pgo JM, Cerqueira J, Sousa N: Chronic unpredictable stress inhibits nociception in male rats. Neurosci Lett 2004; 359:73–6
32. Bardin L, Malfetes N, Newman-Tancredi A, Depoortre R: Chronic restraint stress induces mechanical and cold allodynia, and enhances inflammatory pain in rat: Relevance to human stress-associated painful pathologies. Behav Brain Res 2009; 205:360–6
33. Pedersen LH, Blackburn-Munro G: Pharmacological characterisation of place escape/avoidance behaviour in the rat chronic constriction injury model of neuropathic pain. Psychopharmacology (Berl) 2006; 185:208–17
34. Berna C, Leknes S, Holmes EA, Edwards RR, Goodwin GM, Tracey I: Induction of depressed mood disrupts emotion regulation neurocircuitry and enhances pain unpleasantness, Biol Psychiatry 2010, pp 1083–90
35. Kenntner-Mabiala R, Andreatta M, Wieser MJ, Mhlberger A, Pauli P: Distinct effects of attention and affect on pain perception and somatosensory evoked potentials. Biol Psychol 2008; 78:114–22
36. Pinerua-Shuhaibar L, Villalobos N, Delgado N, Rubio MA, Suarez-Roca H: Enhanced central thermal nociception in mildly depressed nonpatients and transiently sad healthy subjects. J Pain 2011; 12:360–9
37. Gonzalez MM, Aston-Jones G: Light deprivation damages monoamine neurons and produces a depressive behavioral phenotype in rats. Proc Natl Acad Sci U S A 2008; 105:4898–903
38. Narita M, Kaneko C, Miyoshi K, Nagumo Y, Kuzumaki N, Nakajima M, Nanjo K, Matsuzawa K, Yamazaki M, Suzuki T: Chronic pain induces anxiety with concomitant changes in opioidergic function in the amygdala. Neuropsychopharmacology 2006; 31:739–50
39. Suzuki T, Amata M, Sakaue G, Nishimura S, Inoue T, Shibata M, Mashimo T: Experimental neuropathy in mice is associated with delayed behavioral changes related to anxiety and depression. Anesth Analg 2007; 104:1570–7
40. Alba-Delgado C, Llorca-Torralba M, Horrillo I, Ortega JE, Mico JA, Sánchez-Blazquez P, Meana JJ, Berrocoso E: Chronic pain leads to concomitant noradrenergic impairment and mood disorders. Biol Psychiatr; 2012 (in press)
41. Kontinen VK, Kauppila T, Paananen S, Pertovaara A, Kalso E: Behavioural measures of depression and anxiety in rats with spinal nerve ligation-induced neuropathy. Pain 1999; 80: 341–6
42. Matsuzawa-Yanagida K, Narita M, Nakajima M, Kuzumaki N, Niikura K, Nozaki H, Takagi T, Tamai E, Hareyama N, Terada M, Yamazaki M, Suzuki T: Usefulness of antidepressants for improving the neuropathic pain-like state and pain-induced anxiety through actions at different brain sites. Neuropsychopharmacology 2008; 33:1952–65
43. Yalcin I, Bohren Y, Waltisperger E, Sage-Ciocca D, Yin JC, Freund-Mercier MJ, Barrot M: A time-dependent history of mood disorders in a murine model of neuropathic pain. Biol Psychiatry 2011;70:946–53
44. Beckmann M, Johansen-Berg H, Rushworth MF: Connectivity-based parcellation of human cingulate cortex and its relation to functional specialization. J Neurosci 2009; 29: 1175–90
45. Wiech K, Tracey I: The influence of negative emotions on pain: Behavioral effects and neural mechanisms. Neuroimage 2009; 47:987–94
46. Rainville P, Duncan GH, Price DD, Carrier B, Bushnell MC: Pain affect encoded in human anterior cingulate but not somatosensory cortex. Science 1997; 277:968–71
47. Ruscheweyh R, Deppe M, Lohmann H, Stehling C, Flel A, Ringelstein EB, Knecht S: Pain is associated with regional grey matter reduction in the general population. Pain 2011; 152:904–11
48. Wagner G, Koch K, Schachtzabel C, Reichenbach JR, Sauer H, Schlsser Md RG: Enhanced rostral anterior cingulate cortex activation during cognitive control is related to orbitofrontal volume reduction in unipolar depression. J Psychiatry Neurosci 2008; 33:199–208
49. Shackman AJ, Salomons TV, Slagter HA, Fox AS, Winter JJ, Davidson RJ: The integration of negative affect, pain and cognitive control in the cingulate cortex. Nat Rev Neurosci 2011; 12:154–67
50. Cao H, Gao YJ, Ren WH, Li TT, Duan KZ, Cui YH, Cao XH, Zhao ZQ, Ji RR, Zhang YQ: Activation of extracellular signal-regulated kinase in the anterior cingulate cortex contributes to the induction and expression of affective pain. J Neurosci 2009; 29:3307–21
51. Dai RP, Li CQ, Zhang JW, Li F, Shi XD, Zhang JY, Zhou XF: Biphasic activation of extracellular signal-regulated kinase in anterior cingulate cortex distinctly regulates the development of pain-related anxiety and mechanical hypersensitivity in rats after incision. Anesthesiology 2011; 115:604–13
52. Kuipers SD, Trentani A, Den Boer JA, Ter Horst GJ: Molecular correlates of impaired prefrontal plasticity in response to chronic stress. J Neurochem 2003; 85:1312–23
53. Wei F, Zhuo M: Activation of Erk in the anterior cingulate cortex during the induction and expression of chronic pain. Mol Pain 2008; 4:28
54. Rodriguez-Raecke R, Niemeier A, Ihle K, Ruether W, May A: Brain gray matter decrease in chronic pain is the consequence and not the cause of pain. J Neurosci 2009; 29: 13746–50
55. Todorovic C, Sherrin T, Pitts M, Hippel C, Rayner M, Spiess J: Suppression of the MEK/ERK signaling pathway reverses depression-like behaviors of CRF2-deficient mice. Neuropsychopharmacology 2009; 34:1416–26
56. Blackburn-Munro G, Blackburn-Munro RE: Chronic pain, chronic stress and depression: Coincidence or consequence? J Neuroendocrinol 2001; 13:1009–23
57. Ayensu WK, Pucilowski O, Mason GA, Overstreet DH, Rezvani AH, Janowsky DS: Effects of chronic mild stress on serum complement activity, saccharin preference, and corticosterone levels in Flinders lines of rats. Physiol Behav 1995; 57:165–9
58. Grnli J, Murison R, Bjorvatn B, Srensen E, Portas CM, Ursin R: Chronic mild stress affects sucrose intake and sleep in rats. Behav Brain Res 2004; 150:139–47
59. Shi M, Wang JY, Luo F: Depression shows divergent effects on evoked and spontaneous pain behaviors in rats. J Pain 2010; 11:219–29
60. Kant GJ, Leu JR, Anderson SM, Mougey EH: Effects of chronic stress on plasma corticosterone, ACTH and prolactin. Physiol Behav 1987; 40:775–9
61. Rabasa C, Muoz-Abellan C, Daviu N, Nadal R, Armario A: Repeated exposure to immobilization or two different footshock intensities reveals differential adaptation of the hypothalamic-pituitary-adrenal axis. Physiol Behav 2011; 103: 125–33
62. Bomholt SF, Mikkelsen JD, Blackburn-Munro G: Normal hypothalamo-pituitary-adrenal axis function in a rat model of peripheral neuropathic pain. Brain Res 2005; 1044:216–26
63. Vissers K, Adriaensen H, De Coster R, De Deyne C, Meert TF: A chronic-constriction injury of the sciatic nerve reduces bilaterally the responsiveness to formalin in rats: A behavioral and hormonal evaluation. Anesth Analg 2003; 97: 520–5, table of contents